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THE PYROTANNIC ACID METHOD FOR THE QUANTITATIVE DETERMINATION OF CARBON MONOXIDE IN BLOOD AND IN AIR

ITS USE IN THE DIAGNOSIS AND INVESTIGATION OF CASES OF CARBON MONOXIDE POISONING

BY

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THE PYROTANNIC ACID METHOD FOR THE QUANTITATIVE DETERMINATION OF CARBON MONOXIDE IN BLOOD AND IN AIR:[1] ITS USE IN THE DIAGNOSIS AND INVESTIGATION OF CASES OF CARBON MONOXIDE POISONING[2]

By R. R. Sayers and W. P. Yant

INTRODUCTION

Carbon monoxide (CO) may be formed in many places and inhalation of this insidious gas is a frequent and widely distributed cause of poisoning that ranges in severity from headache and lassitude to unconsciousness and death. People are continually being affected by CO in homes and garages, around gas and gasoline engines and blast furnaces, in fighting fires, after blasting in mines and quarries, and after mine fires and explosions; in fact, at any place where there is possibility of exposure to the products of combustion of carbonaceous fuels or products. As cases of this type of poisoning often occur from the most unsuspected sources, and as the indicating symptoms of CO poisoning, such as headache, nausea, dizziness, collapse, and unconsciousness are often attributed to other causes, it is essential to have suitable means whereby the true condition can be ascertained. Quick, accurate determination is not only of value for making diagnoses and giving proper treatment, but for investigating the causes and conditions under which the poisoning occurred, as well as providing means for examining doubtful atmospheres to prevent and guard against the recurrence of poisoning.

The only infallible diagnosis of CO poisoning is made by examination of the blood for carbon monoxide hemoglobin, HbCO, the compound which the gas forms with blood and through which it possesses noxious properties. A mere qualitative examination for this compound will indicate whether or not CO is present, but in view of the obvious desirability of knowing whether or not CO is the primary cause of the condition of the patient, it is necessary to make a quantitative determination of the HbCO present.

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[1] Sayers, R. R., Yant, W. P., and Jones, G. W., The pyrotannic acid method for the quantitative determination of carbon monoxide in blood and air: Repts. of Investigations, Serial 2486, Bureau of Mines, June, 1923, 6 pp.; Public Health Reports, vol. 38, No. 40, 1923, pp. 2311–2320.

[2] Sayers, R. R., and Yant, W. P., Dangers of and treatment for carbon monoxide poisoning: Repts. of Investigations, Serial 2476, Bureau of Mines, June, 1923, 11 pp.; Carbon-monoxide poisoning in homes and industry: Repts. of Investigations, Serial 2593, Bureau of Mines, April, 1924, 7 pp.

Methods have been devised for the quantitative analysis of CO when present in blood and in air in quantities large enough to be dangerous to the health and safety of a person. Some of them are suitable as to accuracy, yet all have some objectionable features in that they require elaborate and expensive apparatus, and special technique and training on the part of the analyst, or are too delicate and cumbersome for field use. The last factor is important in investigations pertaining to the cause, diagnosis, and treatment of this type of industrial and domestic accident.

The Bureau of Mines, in its investigation of many cases of industrial and domestic poisoning from CO, found it necessary to develop a method and apparatus that could be immediately taken to the scene of a poisoning, and which would give accurate results as to the CO in the blood and in the air. The method described herein has been used frequently during the past two years in the investigation of fatal and nonfatal cases of poisoning from gas stoves, automobile exhaust gases, and blast-furnace gas; also in the analysis of air in vehicular tunnels and in mines after blasting and during rescue operations after a mine explosion. It has been used in the laboratory for experimental investigation of CO poisoning of men and animals and has also been used with equal success by other investigators and by corporations both in America and in foreign countries. In all of this work the method has been found very reliable and accurate, and admirably fulfills requirements for the examination of blood and air. Its simplicity and ease of operation make it well suited to the needs of hospitals, industrial surgeons, safety engineers, coroners, departments of public safety, boards of health, and other allied organizations.

QUANTITATIVE DETERMINATION OF CARBON MONOXIDE IN BLOOD

DESCRIPTION OF METHOD

According to Wetzel's qualitative test,[3] when normal blood, diluted 1:4 with water and shaken with an equal volume of 1 per cent tannic acid, is allowed to stand for 24 hours, a gray suspension is formed, whereas blood containing HbCO remains carmine red. This color reaction is sensitive, and when once formed changes so slowly that the authors decided to adapt it to quantitative purposes.

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[3] McNally, W. D., Carbon-monoxide poisoning: Jour. Amer. Med. Assoc., vol. 69, Nov. 10, 1917, pp. 1586–1590.

From two specimens of blood—the one, ordinary venous blood, and the other, blood saturated with CO—solutions (1:9) were made with distilled water and, by use of these, mixtures were prepared varying from 0 to 100 per cent HbCO by 10's (0, 10, 20, etc.). Equal volumes of these mixtures were then put in each of a series of test tubes and treated with tannic acid. When the tubes containing the mixture were arranged in a rack and left standing for 10 hours the graduation of the color change with the amount of HbCO was quite evident. A sample of an "unknown" blood, prepared and similarly conditioned, could be readily matched with a corresponding standard.

The results of the preliminary work promised an accurate yet easy method for quantitative determination of CO in blood, and it was decided to investigate it further to find the best working conditions, such as the concentration of blood and acid, size of test tubes, and lighting most effective for making the comparisons of unknowns and standards. Later the results of using anticoagulants[4] to preserve the blood to be used as material for standards, the storage of blood for standard material, the possibility of decreasing the time required for the formation of the color, the permanency of the prepared standards, and finally the preparation of permanent standards from pigments were included in the investigation.

PROCEDURE FOR MAKING STANDARDS FROM BLOOD

As the result of a rather extensive series of tests of the above conditions the procedure finally adopted for making standards from blood is as follows:

By use of a modified Keidel tube, or any intravenous apparatus, 5 c. c. or more of human blood (animal blood, especially beef blood, may be satisfactorily used if obtainable) is drawn and kept from clotting by the addition of 0.05 gram of potassium citrate, $K_3C_6H_5O_7H_2O$, per 10 c. c. of whole blood. The blood thus obtained is divided into approximately equal parts, one of which is immediately diluted 1 in 10 with distilled water, while the other is saturated with 3 to 5 per cent of CO gas, then diluted 1 in 10 with distilled water. The blood should be saturated with CO before it is diluted with distilled water, in order to minimize the volume of CO gas dissolved in the resulting solution. If the saturation is made after dilution with water, the volume of dissolved gas would be so increased that it would seriously affect the calculated relation when the resulting HbCO solution is mixed with the solution of oxyhemoglobin, O_2–Hb.

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[4] Sayers, R. R., O'Brien, H. R., Jones, G. W., and Yant, W. P., Collection and preservation of blood samples for determination of carbon monoxide: Public Health Reports, vol. 38, No. 35, Aug., 1923, pp. 2005–2011.

From these solutions of approximately all HbO_2 and HbCO respectively, mixtures are made which total 1 c. c., but vary from 0 to 100 per cent HbCO in steps of 10. These solutions are contained in a test tube, approximately five-sixteenths inch inside diameter, of clear, thin, glass. To each standard thus prepared is added 1 c. c. of a mixture consisting of equal parts of a strictly fresh solution of 2 per cent pyrogallic acid and a solution of 2 per cent tannic acid, after which the tube is inverted twice to insure thorough mixing. The tube should be sealed immediately by pouring a little melted paraffin on top of the contents while the tube is immersed in cold water as a caution against overheating. This temporary seal will exclude the air until the walls of the tubes become dry, when a tight, permanent seal can be made by placing a disk of cardboard on top of the paraffin and filling the remainder of the tube with ordinary sealing wax. Care should be taken to exclude all air. Standards thus prepared develop their full color in 30 minutes, and if properly sealed and kept in a cool place will retain their permanence for 1 to 2 weeks, not changing enough to interfere with accuracy of determination.

PERMANENT STANDARDS

Although very reliable for a limited time, the standards prepared from blood are not accurate over long periods. The necessity of having to prepare standards frequently in order to have a set ready for emergencies is undesirable; accordingly permanent standards matching the corresponding blood colors were prepared from pigments (artists' oil colors). Standards thus prepared are ready for use at all times.

DESCRIPTION OF APPARATUS FOR EXAMINATION OF BLOOD

A complete set of apparatus and chemicals for determining CO in blood is listed below and is shown in Plate I, *A*, as assembled in a complete, durable, and compact outfit by the Bureau of Mines for field investigations. For examination of air as well as blood, the additional apparatus shown in Plate I, *B*, is required.

a. Set of color standards, prepared either from blood or pigments, to represent the color of blood having varying known amounts of HbCO (as 0, 10, 20, 30, etc.). The standards are contained in small clear test tubes five-sixteenths inch in diameter and having a volume of 2 c. c., which may be arranged in a suitable rack with spaces between for interposing tubes of similar size that contain the blood samples for analysis.

b. Small test tubes (of the same size and glass as those used for the standards) in which to prepare specimens for analysis.

c. Dilution pipette. The dilution may be made by using a 0.1-c. c. pipette for measuring the blood into the required volume of water or by having a dilution pipette (as shown), with a capillary stem that holds 0.1 c. c. and a

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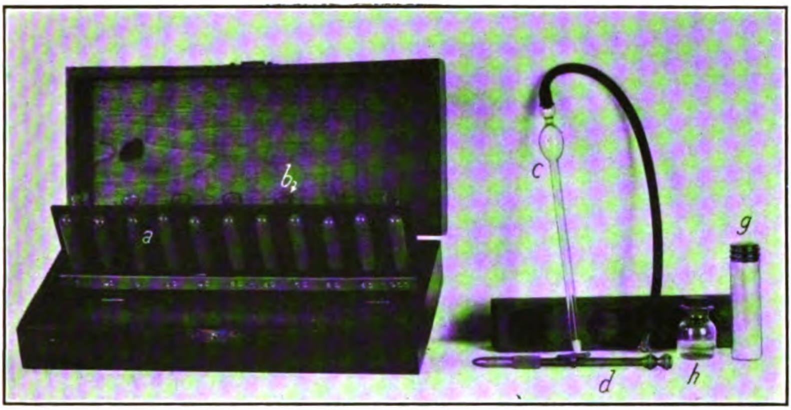
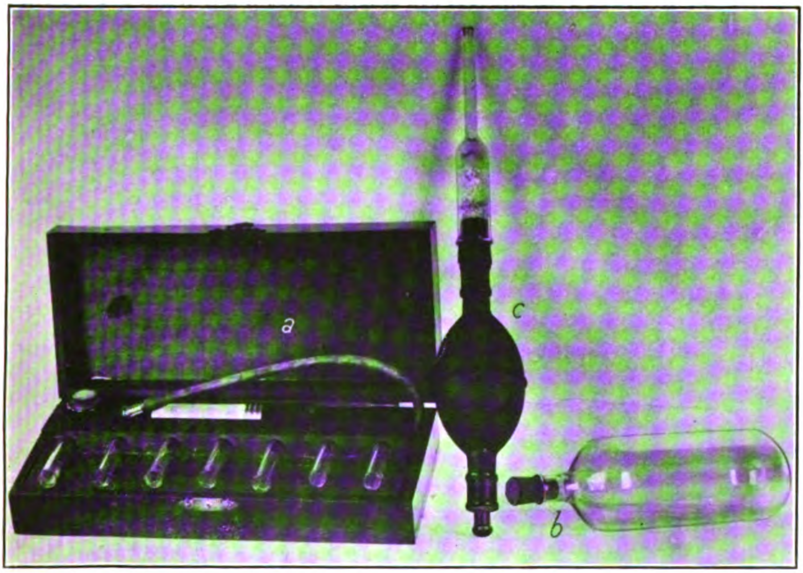
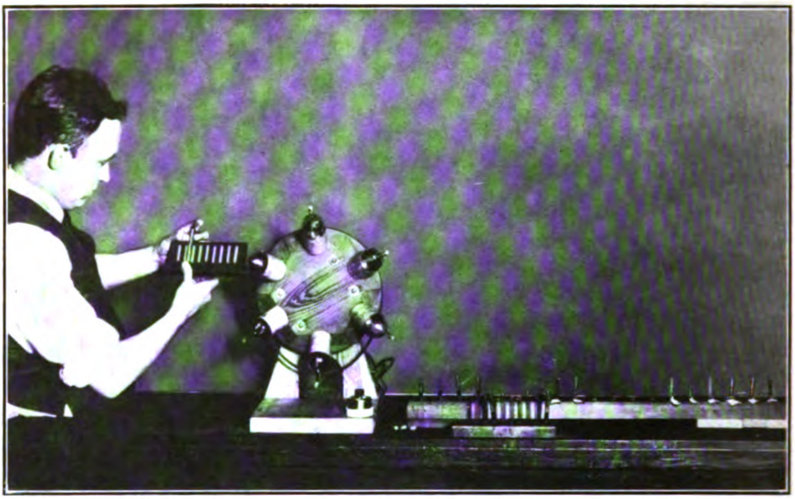


A. APPARATUS FOR DETERMINATION OF CO IN BLOOD SEE TEXT FOR EXPLANATION OF LETTERS



B. APPARATUS FOR SAMPLING AND DETERMINATION OF CO IN AIR. SEE TEXT FOR EXPLANATION OF LETTERS



A. MOTOR-DRIVEN EQUILIBRATOR

B. TAKING SAMPLE OF BLOOD FOR ANALYSIS bulb above that has a total volume of 2.0 c. c., thus making a dilution of 1 in 20, which seems to be the most suitable blood concentration.

d. Spring hemospast or blood lancet for making a small wound in the finger or other convenient place from which the blood may be obtained.

e. Small watch glass or spot plate (not shown) for catching the blood as it flows from the wound. This is not essential, as the blood may be drawn up directly from the bead over the wound; however, the spot plate is convenient where two or more specimens are desired from the same wound, as for air analysis, which will be described later.

f. Piece of one-fourth-inch rubber hose 10 inches long (not shown), or a similar band of rubber for wrapping the finger, beginning at the base and proceeding toward the tip in order to force the blood out if it does not flow freely when the sample is being taken.

g. Mixture of equal parts by weight of tannic and pyrogallic acids either solid or in solution. If in solid form it may be prepared in capsules or tablets, or, if it is in powder form, it may be kept in a bottle as in the equipment used by the Bureau of Mines, with a measuring spoon holding approximately 0.04 gram, which is the amount found to be the most suitable for 2 c. c. of a 1 in 20 blood solution. If less blood solution is used the amount of the acids must be decreased correspondingly.

If a solution of tannic and pyrogallic acids is used, it may be made in concentrated form so that 2 or 3 drops will be equivalent to 0.04 gram of the acids. Another procedure found to work equally well is to make the solution of such strength that 1 c. c. contains 0.04 gram of the mixed acids, in which case the blood specimen is initially diluted 1 in 10. Although solutions work more rapidly they deteriorate quickly, and must be made fresh each day.

h. Bottle of water (preferably, but not necessarily, distilled, if clean tap water is available) for making the blood dilutions.

All of this apparatus can be arranged in a compact pocket case, 8⅞ by 4 by 2¼ inches, as shown in Plate I, *A*, and can be kept in readiness for immediate use.

PROCEDURE FOR SAMPLING AND TESTING BLOOD FOR CARBON MONOXIDE

The following procedure is based on the apparatus described, but it may be modified to suit blood apparatus and facilities at hand, as suggested before:

A small puncture wound (approximately 2 mm. deep) is made with the hemospast in the tip of the finger of the victim, or suspected victim, of CO poisoning, and several drops of blood are caught on the spot plate or drawn directly into the pipette. (Pl. II, *B*.) If the blood does not flow freely, the finger is wrapped with the rubber hose, beginning at the base and progressing toward the tip. Massaging the finger also aids the flow. (If death has intervened, it may be difficult to obtain liquid blood, but this usually can be done during embalming.) When the blood has been procured, it is quickly drawn into the stem of the pipette to the 0.1-c. c. mark. The pipette is then held horizontally, and any blood on the exterior of the tip is removed. The tip is raised slightly to allow a little of the blood to flow into the diluting bulb, and, by inserting it quickly into the bottle of water and using suction at the same time, it can be filled to the 2-c. c. mark to give proper dilution. The blood solution is then discharged into one of the test tubes, a little of the solution being drawn back once or twice into the pipette to wash out any adhering concentrated blood. To eliminate possible clotting, the entire procedure of obtaining the blood specimen should be as rapid as possible. If it is desired to take more than one sample, the pipette can be rinsed out with a little dilution water, care being taken to blow the capillary stem free from water and to dry the tip of the pipette before the next sample is obtained.

Immediately after the blood solution has been discharged into the test tube, approximately 0.04 gram of the tannic-pyrogallic acid mixture is added by the spoon, capsule, or solution method, and the tube is inverted several times to insure thorough mixing with the reagents. The tube is then placed in the rack and allowed to stand 15 minutes at room temperature (if particles of the solid acids settle out, the tube should be inverted several more times), at the end of which period it is compared with the standards by interposing it between them until the standard is found which most nearly matches it. If CO is indicated, the tube should be allowed to stand 15 minutes longer and another reading made. The latter reading should be taken as the more accurate. The percentage of HbCO, or what is designated as "blood saturation," is estimated from the value of that standard.

The observations are best made in daylight, but not in direct sunlight. The observer should stand with his back to the light, viewing the tubes by reflection, and should change their positions several times to note any difference due merely to unequal lighting effect. Observations may be made after several hours without serious loss of accuracy, although this is only advisable in case of necessity.

BLOOD SATURATION AND SYMPTOMS

Symptoms of CO poisoning more or less parallel the blood saturation as determined by the above method. They may be divided into two stages, depending on the action of the nervous system, the first covering the period beginning with normal and ending with syncope; and the second, a paralysis of the central nervous system, beginning with syncope, extending through coma, and ending in apnea. The order of action in each, according to Crile,[5] is the brain, spinal cord, and medulla. The stages are briefly as follows:

Stage 1. Tightness across forehead, dilation of cutaneous vessels, headache (frontal and basal), throbbing in temples, weariness, weakness, dizziness, nausea and vomiting, loss of strength and muscular control, increased pulse and respiration rates, and collapse.

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[5] Crile, G. W., Hemmorrhage and transfusion: Experimantal and clinical research, 1909.

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The same individual seldom experiences all of these symptoms. In some cases the poisoning may proceed to the stage of syncope without the victim feeling any of these symptoms; in fact, it is a frequent occurrence if the poisoning has been rapid.

Stage 2. Increased pulse and respiratory rates, fall of blood pressure, loss of muscular control (especially sphincters), loss of reflexes, coma usually with intermittent convulsions, Cheyne-Stokes' respiration, slowing of pulse, slow and shallow respiration, cessation of respiration, death.

With a given blood saturation, the character and severity of symptoms acquired during exposure depend upon the time required to attain that saturation and the degree of muscular activity—in other words, the extent of oxygen deprivation. The symptoms also depend upon the personal idiosyncrasy and physical condition of the victim.

The symptoms decrease in number with the rate of saturation. If exposed to high concentration, the victim may experience but few (weakness and dizziness) of the symptoms given under stage 1. If a given saturation has been acquired by long exposure to a low concentration, the symptoms and after effects will be a great deal more severe than when a similar saturation has been acquired by short exposure to a high concentration. Activity increases the number and accentuates the character of the symptoms after exposure. A person at rest may pass into a state of dizziness and unconsciousness without experiencing any marked previous effects. After coal-mine explosions the effect of personal idiosyncrasy and physical condition may be noticed. It is common to find groups of men, some of whom will be dead and others conscious, although all have been exposed to the same concentrations of gas for the same period.

The various percentages of blood saturation and the resultant symptoms may be summarized as follows:

Symptoms caused by various percentages of carbon monoxide in the blood

Blood saturation Per cent	Symptoms
0—10	None.
10—20	Tightness across forehead; possibly slight headache.
20—30	Headache; throbbing in temples.
30—40	Severe headache, weakness, dizziness, dimness of vision, nausea and vomiting, collapse.
40—50	Same as previous item with more possibility of collapse and syncope, increased respiration and pulse.
50—60	Syncope; increased respiration and pulse, coma with intermittent convulsions; Cheyne-Stokes' respiration.
60—70	Coma with intermittent convulsions, depressed heart action and respiration, possibly death.
70—80	Weak pulse and slowed respiration; respiratory failure and death.

PATHOLOGY

The most characteristic change is the scarlet-red color of the blood. The color of the skin of persons poisoned by CO often differs from that of persons dying from other causes. Some persons have rose-red spots on the face, neck, breast, and limbs, and others dark red or violet, but the nonappearance of such spots should not be taken as indicating the absence of CO poisoning. The coagulating power of the blood is not changed.[6] Reddening of the digestive tract and peritoneum with echymoses, effusions, or extensive hemorrhages is present in many of the cases. At times degenerative changes are found in the walls of the blood vessels. There are no marked characteristic changes in the respiratory passages, except those due to blood changes; however, it is noteworthy that viscous, thick, frothy mucus, or foreign matter, such as stomach contents, may be found in the larynx, trachea, and bronchi. In the brain hyperemia, edema, and hemorrhages are found in a majority of the cases. The ventricles and arachnoid space contain a copious amount of watery or blood-tinged effusions. Foci of degeneration frequently have been found. The pia of the spinal cord may be markedly hyperemic. Localized edema and punctiform hemorrhages may also be present. Degeneration has been found in the cells of the anterior horn in the cervical and dorsal cord.[7]

AFTER EFFECTS

The after effects of CO poisoning range from practically none to headaches, muscular pains, long periods of unconsciousness, loss of strength, and mental derangement, such as loss of memory, paralysis, and temporary blindness. In nearly all cases these clear up in a day or two, but they have extended over months.

As was true of symptoms, the possibility and severity of these aftereffects depend upon the duration of oxygen deprivation, personal idiosyncrasy, and physical condition of the victim before exposure.

Slight exposures to high concentrations cause far less tissue damage than long exposures to low concentrations, the effect of oxygen deprivation being additive. With some men there is a specific tolerance to this deprivation, and they may experience few or no after effects from exposures which are serious to fellow workmen. It is very probable that much of this idiosyncrasy is due to previous health condition, and that many diseases, such as cerebral arterio-scelerosis, syphilis, and acute alcoholism, are contributory to serious after effects.

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[6] Forbes, H. S., and Hompe, Louise, Carbon monoxide, illuminating gas, and benzol; their effect on blood coagulation time: Jour. Ind. Hyg., vol. 3, Nov., 1921, pp. 213–216.

[7] Lewin, L., Die Kohlenoxydvergiftung. Berlin, 1920, 269 pp.

PREVENTIVE MEASURES

The chief factors in preventing CO poisoning are as follows: (1) Good ventilation, as this will dilute and carry away the gas; (2) avoidance as much as possible of any exposure to air known to contain CO; (3) use of adequate protective equipment when atmospheres known to contain CO are to be encountered; (4) keeping calm when it is necessary to enter or be exposed unprotected to atmosphere containing the gas, not hurrying but getting to fresh air as quickly and with as little exertion as possible.

EMERGENCY TREATMENT

In the treatment of CO poisoning the most important thing is to get that gas out of the blood as rapidly as possible, thus relieving the anoxemia (oxygen-want in the tissues) and decreasing the possibility of serious after effects or even loss of life through failure of the heart and respiration.

As soon as a victim begins to breathe pure air, the tension of the CO in the pulmonary air is less than the tension of the CO in the blood, and a great deal of the CO which is liberated by the dissociation of the HbCO into CO and Hb diffuses from the blood into the lungs and is exhaled, thus beginning a reversal of the process of poisoning; the blood will then gradually resume its normal oxygen-carrying function. However, this normal elimination is too slow, requiring 8 to 15 hours to reduce the HbCO to 10 per cent of the total Hb, and often results in serious effects.

The two main factors accelerating this reaction are: (1) To increase the amount of oxygen in the pulmonary air and plasma, since the reaction is one of mass action; and (2) to increase the lung ventilation, which will remove faster the CO given up by the blood to the lungs. Inhalations of pure oxygen will remove the CO practically four times as fast as will normal air,[8] while a mixture of 5 per cent of CO_2 in O_2 will cause increased lung ventilation[9] and remove the CO five to six times as fast as normal air. The advantage in the use of O_2 alone is that it is more available, owing to its use in the industries for other purposes. Both of these inhalation treatments are given preferably with an inhaler, but the O_2

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[8] Sayers, R. R., and Yant, W. P., The elimination of carbon monoxide from blood by treatment with air, with oxygen, and with a mixture of carbon dioxide and oxygen: Public Health Reports, vol. 38, No. 36, 1923, pp. 2053–2074.

[9] Henderson, Yandell, and Haggard, H. W., The elimination of carbon monoxide from the blood after a dangerous degree of asphyxiation, and a therapy for accelerating the elimination: Jour. Pharm. and Exp. Therap., vol. 16, 1920, pp. 11–20.

or O_2–CO_2 mixture may be given by improvised apparatus or sprayed directly from the tank over the patient's face when an inhaler is not at hand. Caution should be observed, however, to open the valve and regulate the gas flow before turning it in the direction of the patient; moreover, no improvised mask or device should be used in which the pressure can build up, thereby injuring the patient. It should be emphasized that an inhaler is much to be preferred, because it is far more efficient than any improvised apparatus for the administration of these gases.

ACUTE POISONING

The steps in effective treatment of acute CO poisoning are:

1. The victim should be removed to fresh air as soon as possible.

2. If breathing has stopped, is weak and intermittent, or present in but occasional gasps, artificial respiration by the Schaefer method[10] should be given persistently until normal breathing is resumed, or until after the heart has stopped.

3. Pure oxygen, or a mixture of 5 per cent of CO_2 in O_2, should be administered, beginning as soon as possible and continuing for at least 20 minutes in mild cases and as long as 1 to 2 hours if necessary in severe cases if the patient does not regain consciousness.

4. Circulation should be aided by rubbing the limbs of the patient and keeping the body warm with blankets, hot-water bottles, hot bricks, or other devices, care being taken that hot objects are wrapped or do not come in contact with the body and produce burns. This aids in tiding the body over a period of low vitality. Other stimulants, such as hypodermics of caffein, sodium benzoate, or camphor in oil, should be administered only by a doctor after he has considered the possibility of collapse following excessive stimulation.

5. The patient should be kept at rest, lying down in order to avoid any strain on the heart; later he should be treated as a convalescent and given plenty of time to rest and recuperate.

6. After effects of CO poisoning should be treated symptomatically.

In conclusion, it should be emphasized that inhalations, for a period of 20 to 30 minutes, of oxygen or a 5 per cent mixture of CO_2 in O_2, if available, will, when given immediately, greatly lessen the number and severity of symptoms of CO poisoning, as well as decrease the possibility of serious aftereffects. All industries in which this type of poisoning is common should provide apparatus (inhalers) for the efficient administration of these treatments. This apparatus should be placed at points most convenient for treating CO poisoning, and employees should be trained in its use, so that resuscitation may be effected as soon as symptoms of poisoning are noted.

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[10] Manual of first-aid instruction for miners, revised by R. R. Sayers: Bureau of Mines, 1922, pp. 33–35.

QUANTITATIVE DETERMINATION OF CARBON MONOXIDE IN AIR

REACTIONS OF BLOOD WITH CARBON MONOXIDE-AIR MIXTURES

When a blood solution is exposed to air containing CO, there is competition between the CO and the O_2 for the hemoglobin, the competing or combining power (reaction constant) of the former being approximately 300 times that of the latter. If the exposure lasts long enough, an equilibrium will be established between the competing forces, the amount of hemoglobin combining with each depending upon the product of its competitive power times the respective partial pressures of CO and O_2 in the gas mixture. Thus, by determination of the amount of hemoglobin combining with either of the gases (CO or O_2), and with a knowledge of the partial pressure of one of them, it is possible to calculate the partial pressure of the other. This phenomenon has long been known, and has recently been described by Prince,[11] but this method has been little utilized quantitatively, owing to some objections to the methods for determining HbCO; however, with the apparatus described in this paper, the HbCO can be easily determined, hence the CO content of a CO-air mixture.

DESCRIPTION OF APPARATUS

Suitable apparatus for this method is shown in Plate I, *B* (p. 4), and consists of the following parts (any modification accomplishing the same end may be used):

a. Complete set of standards, as shown in Plate I, *A* (*a*).

b. Air-sample bottles of at least 250 c. c. capacity, fitted with rubber stoppers.

c. Rubber aspirator bulb with attached scrubber (tube of soda lime) for removing gases which might have an interfering effect.

SAMPLING AIR

Samples are obtained by inserting the glass tube on the end of the scrubber into the sample bottle and expirating the air through the sample bottle long enough to purge it of its original contents; this requires at least 25 squeezes of the bulb. The last bulb of gas should be expelled through the sample bottle while the glass tube is being removed, after the removal of which the rubber stopper should be inserted quickly and tightly. If these sample bottles and bulbs are not available the samples may be collected in any ordinary bottle having a capacity of at least 250 c. c. The bottles can be filled with water and then emptied at the place of sampling, the walls being well drained. The samples can then be transported to the place for making the analysis, usually away from the immediate place where the sample has been taken.

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[11] Prince, A. L., The combination of carbon monoxide and hemoglobin and its analytical application: Report of New York State Bridge and Tunnel Commission, Sec. 9, appendix 4, 1921, pp. 188–191.

BLOOD FOR MAKING ANALYSIS

The blood to be used in making the analysis may be taken from a stock solution of relatively fresh human or animal blood, or from a small puncture wound in the tip of the finger (as previously described) of a person who has not been exposed to CO. Dilution is made 1 in 20, as described for analysis of the blood; 2 c. c. of blood solution is then introduced into the sample bottle, manipulation being such as to allow as little gas as possible to escape. The solution may be previously measured out into the small test tubes or introduced directly from the dilution pipette.

EQUILIBRATION

Equilibrating should be done in a rather dimly lighted place. After the stopper has been tightly replaced, the bottle is held horizontally and rotated constantly for 15 to 20 minutes, avoiding violent shaking and agitation. As much as possible of the surface of the bottle should be covered with the blood solution. Every now and then the solution can be centrifugally thrown from the sides to the bottom of the bottle by a quick swinging motion, which allows a new surface to be formed and aids in reaching equilibrium. When a great many samples have to be analyzed, a motor-driven equilibrator, as shown in Plate II, *A* (p. 5), will save time. On this six sample bottles are held at 90° angle from a revolving vertical disk, the speed being such as to allow the solution to flow over the inner surface of the bottles. This apparatus is not essential, however, and would be needed only in making a large number of analyses.

ANALYSIS

When equilibrating has been finished, the solution is poured back into the test tube, the tannic-pyrogallic acid added, and the comparison of colors and determination HbCO made according to the procedure already described.

CALCULATION OF RESULTS

When equilibrium has been established between CO and O_2 Hb, the amount of Hb combined with each (as has been stated) pends on the product of the competitive power or affinity (a) times the partial pressure (p), and the ratio of the amounts can be expressed by the following equation:

$$\frac{\text{Amount combining with CO}}{\text{Amount combining with O}_2} \text{ or } \frac{\text{HbCO}}{\text{HbO}_2} = \frac{\text{pCO} \times \text{aCO}}{\text{pO}_2 \times \text{aO}_2} \quad (1)$$

In the use of equation (1) for the determination of pCO it is necessary to know all the other members, at least to a degree of accuracy that will not affect the results beyond the desired limits of the method. Fortunately, the equation allows much variation without serious affects upon the results, and on that basis average figures can be selected which serve as constants for all practical purposes. A brief discussion of each follows:

HbCO. HbCO is determined by the prescribed method of blood analysis to a degree of accuracy affecting the results obtained with an error of ±0.005 in the region of 0.01 per cent CO, and slowly increasing to ±0.02 in the region of 0.15 per cent CO.

HbO_2. The HbO_2 content is obtained by difference: $100\text{–HbCO}=HbO_2$.

pO_2. The amount of oxygen in normal air is 20.93 per cent by volume, or 2,093 parts per 10,000. Obviously this will be decreased, depending upon the source of contamination with CO. However, unless the amount present falls below 19 per cent, or 1,900 parts per 10,000, the results obtained for pCO will be well within the error incurred through the analysis for HbCO. Even with lower percentages of O_2, the results will not be invalidated from the standpoint of health investigation. If determinations are made for oxygen, or some knowledge (from flame test, etc.) is had of the approximate amount present, these values can be used for pO_2 in the equation; otherwise, 20.93 per cent can be taken as a constant.

$aO_2=1$. Affinity of oxygen is taken as unity.

aCO. On the basis of $aO_2=1$, the aCO is, according to Prince,[12] approximately 300. Although there is a variation with different individuals, that variation could be 50 without seriously affecting the method, the error incurred being approximately ±0.0015 in the region of 0.01 per cent, and ±0.015 in the region of 0.10 per cent CO.

Thus we can substitute these values in equation (1) which becomes:

$$\frac{\text{HbCO}}{100-\text{HbCO}} = \frac{\text{pCO} \times 300}{2093 \times 1}, \quad (2)$$

and solving for pCO we get

$$\text{pCO} = \frac{\text{HbCO}}{100-\text{HbCO}} \times \frac{2093}{300}, \quad (3)$$

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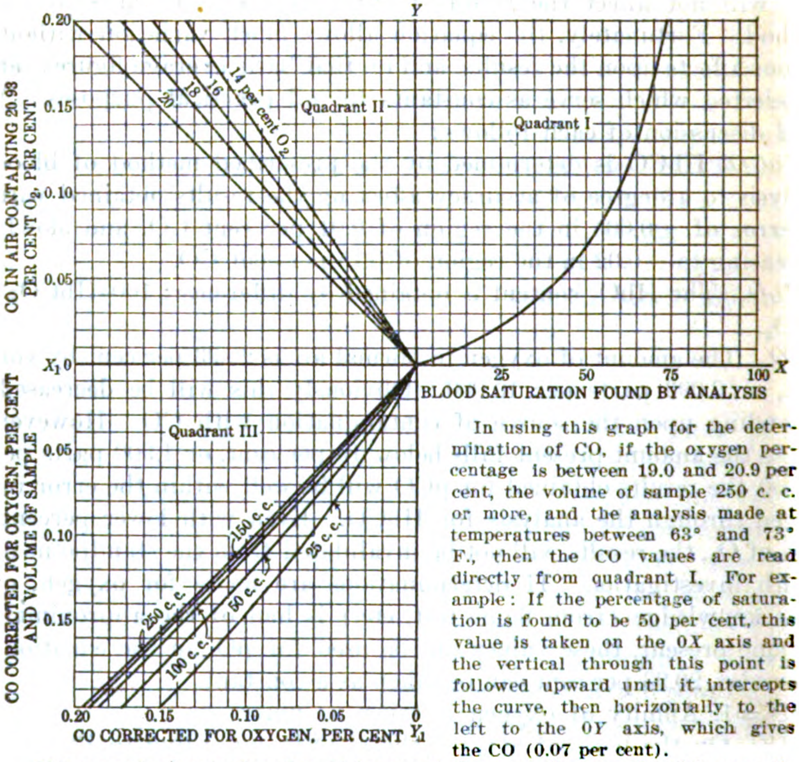
[12] Prince, A. L., work cited.

from which the actual value of pCO can be obtained by substituting the value of HbCO obtained by analysis. For example: A blood solution equilibrated with a gas sample was found to contain 50 per cent of HbCO by comparison with the standards. Substituting this value in equation (3) gives the following:

$$\text{pCO} = \frac{50}{100-50} \times \frac{2093}{300} = 7 \text{ parts per 10,000, or 0.07 per cent.}$$



In using this graph for the determination of CO, if the oxygen percentage is between 19.0 and 20.9 per cent, the volume of sample 250 c. c. or more, and the analysis made at temperatures between 63° and 73° F., then the CO values are read directly from quadrant I. For example: If the percentage of saturation is found to be 50 per cent, this value is taken on the 0X axis and the vertical through this point is followed upward until it intercepts the curve, then horizontally to the left to the 0Y axis, which gives the CO (0.07 per cent).

If the sample contains less than 19.0 per cent of oxygen (for example, 18.0 per cent), correction is made by following the horizontal line through the 0Y axis into quadrant II until it intercepts the curve representing the oxygen percentage that the sample contains. The vertical line passing through this point is followed down to where it intercepts the 0X axis, which gives the corrected percentage of CO (0.06 per cent).

If the sample volume was less than 250 c. c. (for example, 50 c. c.), the vertical line is extended into quadrant III until it intercepts the curve representing the volume of the sample, then horizontally to the right until it intercept the 0Y axis, which gives the corrected percentage of CO (0.077 per cent).

FIGURE 1.—Curves for calculating the percentage of carbon monoxide from the percentage of blood saturation

In order to simplify the operation of making calculations and applying corrections for CO in air, the curves shown in Figure 1 were prepared. These graphically represent the calculations and corrections; and by following the instructions given thereon, the corrected result of CO in air for the blood saturation found can be easily and quickly determined.

CORRECTIONS

TEMPERATURE EFFECT ON DISSOCIATION CURVE FOR CARBON MONOXIDE

Temperature changes the dissociation constant ($aCO=300$); but if the variation does not exceed ±5° F. from 68° F., no correction need be made. To avoid having to make a correction for temperature the equilibrating should be done within these limits.

CORRECTION FOR LOSS OF CARBON MONOXIDE THROUGH COMBINATION WITH HEMOGLOBIN

As there has been some loss of CO from the CO-air mixture, due to CO being taken into combination with the hemoglobin, the results obtained do not represent the CO content in the original sample of gas. If the sample of gas is small (250 c. c. or less), there is a slight error introduced by the CO going into combination with the Hb, which can be corrected by assuming 0.0185 c. c. per 0.1 c. c. of blood as the average amount of CO in combination with Hb when the latter is completely saturated; then from the percentage saturation found by analysis, a pro rata calculation can be made which will serve to correct the results obtained for the CO in the sample of gas. For example, if 37 per cent is found to be the percentage of HbCO in a specimen of blood, it would signify that the blood was in equilibrium with 0.04 per cent of CO in air; but since 0.37×0.018 or 0.007 c. c. was lost to the Hb, if the volume of the original sample was 100 c. c., in reality it contained $0.04+\frac{(0.37\times0.018\times100)}{100}$, or 0.047 per cent CO; or if the sample was 200 c. c., it contained 0.043 per cent. It is recommended that 250-c. c. samples be used, which will render this correction negligible.

ACCURACY OF METHOD

To test the accuracy of the method as outlined, synthetic CO-air mixtures varying from 0.01 to 0.20 per cent were carefully made by volume, and 150-c. c. samples were taken for blood analysis. At some of the higher percentages samples were also collected for combustion analysis by the Haldane method.[13] The blood analyses were run in triplicate, using blood from a different subject for each analysis.

Observations as to the amount of HbCO were made by three men, one experienced and two inexperienced, the latter having no knowledge of the origin of the sample. The results obtained are shown in the following table:

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[13] Burrell, G. A., and Seibert, F. M., The sampling and analysis of mine gases and natural gas: Bull. 42, Bureau of Mines, 1913, 116 pp.

Agreement of results obtained and comparison with combustion analyses

Per cent of CO by tannic acid method					Calculated from gas volumes	By combustion analyses
Observer			Maximum variations	Average		
1	2	3				
0.000	0.000	0.000	0.005	0.001	0.000	--------
0.000	0.000	0.005				
0.000	0.000	0.000				
0.000	0.005	0.008	0.005	0.009	0.010	--------
0.012	0.010	0.008				
0.010	0.005	0.010				
0.020	0.025	0.025	0.010	0.021	0.020	--------
0.020	0.020	0.020				
0.025		0.020				
0.035	0.040	0.045	0.010	0.038	0.030	0.045
0.035	0.035	0.035				
0.040	0.040	0.040				
0.065	0.050	0.065	0.020	0.054	0.050	0.060
0.050	0.050	0.050				
0.065	0.045					
0.080	0.080	0.065	0.025	0.077	0.070	--------
0.080	0.090	0.065				
0.140	0.140	0.120	0.030	0.120	0.150	0.150
0.110	0.115	0.120				
0.17	0.16	0.18	0.030	0.180	0.200	0.190
0.19	0.19	0.18				

From the table it is evident that the agreement between the results obtained by different observers when using blood from different subjects is very good. When no CO was present, out of nine observations on three samples, only one reported it as present, and then only 0.005 per cent. The greatest percentage error reported was that in the case in which 0.000 per cent CO was recorded by one observer for one of the samples when 0.010 per cent was present. As the percentage of CO increased, the absolute error also increased, as would be expected from the nature of the dissociation curve; but in all events the results obtained are within the limits of error allowable from the standpoint of the purpose for which the method was devised.

EXAMPLES OF PRACTICAL USE

During the past two years the method described has been successfully and satisfactorily used in the United States and in foreign countries for the analysis of blood and also the air for CO in investigations of cases of poisoning resulting from exposure to automobile and gas-engine exhaust gases, blast-furnace and producer gas, and gas from stoves and in mines; also for health surveys, laboratory investigations of men and animals, and hospitals. A few typical examples of these follow:

1. Investigation was made where a gas stove was suspected of having caused the death of two persons. The stove was tested in the same room and operated as nearly as possible under the same conditions as when the accident occurred. Samples of gas were analyzed at intervals on the spot, by the method described, and other samples were collected for laboratory analysis by the combustion method. In addition, canaries and white rats were placed in the room and the blood of the latter was analyzed at the completion of the test. The results were as follows:

Tests of blood of rats

Time	Carbon monoxide (per cent)	
	Sample analyzed on the spot by pyrotannic method	Sample taken to laboratory for combustion analysis
Start:		
11.45 a. m.	0. 08	0. 08
1.15 p. m.	. 08	. 08
2.15 p. m.	. 13	. 15
3.15 p. m.	. 19	. 19

Analysis of blood of rats (unconscious), 60 per cent.

One canary died at 3.00 p. m., the other canary was taken out.

2. A factory was investigated where several men were nauseated and overcome. Analysis of blood from these men showed the presence of CO. After search for the cause, a defective exhaust pipe leading from a gas engine to the basement was found.

3. The blood of victims of a leaky blast furnace, when analyzed, showed CO and treatment was given.

4. Analyses of the blood of garage workmen for CO were made in connection with a health survey.

5. The method, due to its accuracy in low concentrations, worked admirably in keeping a check on the fresh air in rescue and recovery operations after a mine explosion to guard against possible contamination and the resulting disagreeable effects. It was also used for the determination of CO in air coming from areas suspected of being on fire.

6. Analysis of air for CO was made to determine how soon men could safely return to the working face in mines after blasting.

7. Analyses were made of the blood of a man supposedly killed by blast-furnace gas. The saturation found was 80 per cent, which confirmed the cause, and no doubt saved much of the time and money ordinarily spent in legal proceedings.

8. Investigations were made of numerous cases of sickness and fatigue of housewives and families, the cause of which was found to be CO from gas appliances. The following case is typical: A man and wife suddenly became ill one evening after dinner, having severe headaches with vomiting. They suspected the food and took treatment accordingly. By morning the illness was worse, and the following evening the woman became unconscious and was taken to the hospital. An examination of the man, made immediately, showed his blood to be 35 per cent saturated with CO, and the air directly from his gas heater contained 0.16 per cent CO, by volume. The weather was cold and gas pressure was low, so both persons sat close to the stove, breathing the products of combustion. A son who was confined to a bed near a door and in another part of the room suffered no ill effects. So convinced was the family that the food was at fault that they submitted samples for analyses; however, with the removal of the stove, further trouble was eliminated.

9. Examination was made of the blood of crane and locomotive workmen who became ill while engaged in clearing wreckage from a railroad tunnel. In this case the results for CO were nearly all negative, most of the illness being caused from heat, humidity, and fatigue. Analysis made of the air indicated no CO, thus confirming the results.

10. Examination was made of men who reported illness, supposedly from CO, while working around gas engines and steel furnaces, mines, garages, etc. The claims of CO being the source were easily substantiated or disapproved, and recommendations were made accordingly.

The above are but a few of the applications of the method for the investigation, diagnosis, and treatment of cases of CO poisoning. Its use should be of great assistance to doctors, coroners, first-aid men, departments of public safety, hospitals, and first-aid organizations in investigating cases of these or allied types, not only so that they may give diagnosis and proper treatment but to aid them in their inquests, insure just decisions on claims, and assist in investigating sources of the trouble.

SUMMARY

A method found to be satisfactory for quantitative analysis of CO in blood and air cases of CO poisoning is outlined.

A compact field apparatus is described. Owing to its durability, and the ease with which accurate and dependable results can be obtained, it should be of great use in investigations pertaining to the cause, diagnosis, and treatment of CO poisoning.

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